Molecular Immunology 100 (2018) 126–135

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

The molecular allergology of subtropical grass pollen

Thina Kailaivasan[a], Janet M. Davies[a,b,*]

[a] School of Biomedical Sciences, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane QLD, Australia
[b] Metro North Hospital and Health Service, Brisbane QLD, Australia

ARTICLE INFO

Keywords:
Grass pollen
Allergy
Allergic rhinitis
Subtropical grass pollen
Bermuda grass pollen
Bahia grass pollen

ABSTRACT

Grass pollens are amongst the most important aeroallergen sources world-wide triggering allergic rhinoconjunctivitis and asthma in sensitised patients. Much of what we know about the allergen components of grasses is informed by research on pollen of temperate (Pooideae) species that are abundant in the temperate climate zones. However, climate changes are altering the biogeographical distribution as well as timing and allergenicity of grass pollens. This provides an impetus for better understanding of the contribution of subtropical subfamilies of grasses to pollen allergy globally. Pollen of Chloridoideae (e.g. *Cynodon dactylon*; Bermuda grass) and Panicoideae (e.g. *Paspalum notatum*; Bahia grass or *Sorghum halepense*; Johnson grass) subfamilies are clinically important in subtropical zones of Australia, Asia, India, Africa, and America. These grasses differ ecologically and phylogenetically from temperate grasses and, importantly their allergen composition is qualitatively different. For example, subtropical grass pollens appear to lack the major group 5 grass pollen allergen family. In this review we summarize current knowledge of the epidemiology and immunology of subtropical Chloridoideae and Pancoideae pollen allergens, describe the biochemical characteristics of known isoforms and variants as well as properties and structures of subtropical pollen allergen components. Whilst only one subtropical allergen component; Cyn d 1 of Bermuda grass pollen, is available commercially for diagnostic use, in a natural purified form, a number of allergens of Panicoideae grass pollen; Zea m 1, Zea m 3 and Zea m 13 of maize, Pas n 1 and Pas n 13 of Bahia, as well as Sor h 1, Sor h 2, Sor h 13 and Sor h 23 of Johnson grass, have been discovered. Research effort is directed towards making available subtropical grass pollen allergen components as innovative treatment and diagnostic options that more specifically address the needs of patients from warmer regions of the globe.

1. Biogeography, epidemiology and immunology of subtropical grass pollen

There is an inverse biogeographical distribution of temperate and subtropical grasses with the subtropical species being more abundant closer to the equator (Esch, 2004). The size of the world's population living in subtropical climates is increasing globally and the subtropical climate zones are widening (Gupta, 2002; Seidel et al., 2008). In southern United States of America (USA), such as Florida, Texas, Louisiana and Mississippi, the population increased by 18.3%–52.3 million between 2000 and 2010 (US Census Bureau). The biomass of subtropical grasses (Morgan et al., 2011) and their range is predicted to expand with climate change (Gupta, 2002), increasing the exposure to subtropical GP allergens and intensifying the burden of allergic respiratory diseases (Beggs, 2009; Ziska and Beggs, 2011). The epidemiology of subtropical grass pollens and their contribution to allergic rhinoconjunctivitis and asthma in subtropical regions has previously been reviewed (Davies, 2014). Here a comparison between temperate and subtropical grass pollen biogeography, epidemiology and immunology is summarized.

In 1972, Hensel and Griffith (1972) examined sensitisation frequencies in the 429 patients from Louisiana, a subtropical region in USA; Bahia (*Paspalum notatum)* GP was the most frequently recognized GP but the patterns of sensitization included SPT positivity to Bahia, Dallis (*Paspalum dilatatum*), Johnson (*Sorghum halepense*), Bermuda (*Cynodon dactylon*) and Timothy (*Phleum pratense)* GP. Application of the Prauss Kausner test (Cohen and Zelaya-Quesada, 2002) with serum of five Bahia GP-allergic patients to non-allergic recipients, showed higher SPT reactivity to Bahia than other species of GP (Hensel and Griffith, 1972).

A survey of sensitisation of 345 children of military personnel with allergic diseases in Lackland, Texas, Bahia GP showed the highest frequency (38%) of positive SPT of a panel of 51 common aeroallergens (Calabria and Dice, 2007). Interestingly, the frequency of sensitivity to

* Corresponding author at: Centre for Children's Health Research, Queensland University of Technology, 62 Graham Street, South Brisbane QLD 4101, Australia.
E-mail address: j36.davies@qut.edu.au (J.M. Davies).

https://doi.org/10.1016/j.molimm.2018.03.012
Received 12 March 2018; Accepted 19 March 2018
Available online 31 May 2018

pollens of other grasses; Bermuda (35.9%), Ryegrass (*Lolium perenne*) (34.8%) and Timothy (34.2%), were only slightly lower. The authors considered the study cohort to be from a "mobile" population and it included children up to 18 years of age. There was an increase in prevalence of allergic sensitivity with age, therefore it was unclear whether sensitisation to GP occurred whilst the children lived in Texas and were exposed to grasses of Texas.

Examination of serum IgE reactivity to GP in allergy patients from Europe and North America revealed high correlations between specific IgE to various GP in single point tests (Andersson and Lidholm, 2003; Johansen et al., 2009). However, the degree of correlation between specific IgE concentrations to subtropical GP and temperate GP was not as high as amongst temperate GP. Most of the patient sera examined in those studies were sourced from patients in regions primarily exposed to temperate GP.

IgE cross-inhibition assays can be used to indicate the avidity of interaction based on the inhibitor concentration at which 50% (IC50) of IgE reactivity with the target allergen is blocked, and the degree of specificity of response (maximum IgE cross inhibition) (Aalberse, 2007). IgE cross reactivity between subtropical and temperate GP is incomplete and mostly non-reciprocal, depending on the origin of the patient and their exposure to subtropical and/or temperate GP allergens (White and Bernstein, 2003; Weber, 2003; Davies et al., 2011a; Davies et al., 2012). The capacity for the immune system to differentiate between allergens of temperate and subtropical GP at the T and B cell level has important implications for the specificity of GP allergy diagnosis and the likely efficacy of GP allergen specific immunotherapy (Nony et al., 2015; Burton et al., 2002; Etto et al., 2012; Eusebius et al., 2002).

In a cross-inhibition study from Minnesota, USA, with pooled sera of five donors highly allergic to northern GP, Bermuda GP was unable to inhibit 50% of IgE with June (*Koeleria macrantha*), orchard (*Dactylis glomerata*), meadow fescue (*Festuca pratensis*) or Ryegrass GP. In the converse experiments, four orders of magnitude more Timothy GP extract was required to achieve 50% inhibition of IgE with Bermuda GP (Leiferman and Gleich, 1976).

Similarly, northern GP showed reciprocal cross-inhibition of IgE reactivity with an array of temperate GP in studies with pooled sera from US army volunteers (Martin et al., 1985). However, Bermuda and Bahia GP extracts showed limited capacity to inhibit pooled serum IgE reactivity with pollens of Ryegrass and Timothy GP extracts in radio allergosorbant assays. Pollen of "northern" grasses inhibited most but not all IgE reactivity with Bahia grass, suggesting "unique allergenicity" for Bahia compared with a temperate GP. Although the origin of the subjects and their primary exposure to temperate or subtropical grasses was not described, distinct IgE reactivity between subtropical and temperate GP were evident.

Small numbers of patients from Florida showed no *in vivo* cross-reactivity between pollen of Timothy and Bahia GP in nasal provocation tests in patients with allergic sensitivity to either Timothy and Bahia GP (Phillips et al., 1989).

Recently, Ramirez et al. (2015) used an allergen challenge chamber in Texas to investigate sensitivity to Timothy GP where subtropical grasses predominate without natural Timothy GP exposure. Of the 22 participants, SPT and specific IgE to Timothy and subtropical Bahia, Johnson and Bermuda GP correlated with symptom scores following exposure to Timothy GP in the allergen challenge chamber. The authors did not observe differences between symptom scores of participants who were locals of Texas and those who had lived outside of Texas for over 5 years. However, at excessively high allergen exposure in the chamber (over 3000 grains per meter cubed over three hours) those participants who were local Texans showed slower kinetics of symptom escalation than those who had been exposed to Timothy GP outside Texas suggesting differences in allergic sensitivity between the two subgroups of participants (Ramirez et al., 2015).

In different states of Australia, Davies et al. (Davies et al., 2011a; Davies et al., 2012) showed that levels of specific IgE reactivity with subtropical and temperate GP differ depending on the biogeographical origin and grass exposure patterns of the patient. Moreover, the finding of specific IgE recognition of subtropical species of GP in patients from subtropical Queensland was subsequently confirmed in separate studies (Nony et al., 2015).

2. Biology and biochemistry of subtropical GP allergens

GP allergen families include proteins with particular biochemical structures and functions within the pollen from which they are derived. The allergens discovered within subtropical GP include the group 1; β-expansin, group 13; polygalacturonase, and others described below. Notably, the highly allergenic group 5 allergen of temperate GP does not occur in subtropical grass pollens. Table A1 summarizes the subtropical GP allergens identified to date for which there is evidence of patient IgE reactivity or allergenicity. Proteomic analysis of Bermuda GP revealed eight allergens that shared similarities to known pollen allergen families based on mass spectrometry and databases comparisons (Kao et al., 2005). Analysis of the complete proteome, transcriptome and allergome of Johnson GP, demonstrated that there are more gene transcripts present within the pollen that encode for allergen-like proteins, and more detectible isoforms translated into proteins and packaged within pollen, than there are IgE-binding proteins detected with serum of relevant, clinically-affected allergy patients. Thus the allergen status of putative allergens identified by molecular biological techniques must be verified (Pomes et al this issue).

2.1. Major allergens: group 1

The major Group 1 GP allergens, β-expansins constitute up to 10% of total pollen (Drew et al., 2011). Functionally, these non-proteolytic glycoproteins are involved in the loosening of the cell walls to facilitate invasion of the pollen tube (Cosgrove et al., 1997). In molecular and biochemical studies of the maize (*Zea mays*) allergen Zea m 1, it was proposed that β-expansins loosens plant cell walls by disrupting noncovalent junctions between the matrix polysaccharide glucuronoarabinoxylan, that binds cellulose (Wang et al., 2016). Cyn d 1, from Bermuda GP, was the first group 1 allergen to be characterized as a 32 kDa protein with high N-terminal sequence homology to the well characterized group 1 allergen of Ryegrass, Lol p 1 (Shen et al., 1988; Matthiesen et al., 1991). Group 1 allergens of subtropical GP show the highest frequency of IgE reactivity by immunoblotting in GP allergic patients (Davies, 2014), ranging from IgE reactivity to Cyn d 1 in 76% of 21 patients from Taiwanese and 100% of 44 patients from New South Wales, Australia (Shen et al., 1988; Ford and Baldo, 1987). A 33 kDa acidic (pI 6.59) allergen purified biochemically from Bahia GP showed IgE reactivity with sera from patients in Florida USA (Ghobrial et al., 2002). The Bahia GP Group 1 allergen of 29 kDa was subsequently cloned, Pas n 1 (Davies et al., 2008; White et al., 2009). By ELISA, recombinant Pas n 1 showed IgE with 47 of 55 (85%) patients from the temperate climate city of Melbourne Australia (Davies et al., 2008) and by ImmunoCAP purified Pas n 1 showed IgE reactivity with 91.2% of 182 GP-allergic (Timbrell et al., 2014). Sor h 1 was identified (Avjioglu et al., 1993) and latter characterized as 30 kDa protein that showed by ELISA IgE reactivity with 76% in sera of 64 patients from Queensland, Australia (Campbell et al., 2015).

2.2. Group 13 allergens

The next prominent group of allergens in subtropical GP are the Group 13 allergens. Zea m 13 was the first subtropical group 13 allergen identified, as a 50–60 kDa protein that had 42% IgE reactivity in 24 GP-allergic patients (Petersen et al., 2001). Digestion of Zea m 13 with endoproteinase Lys-C and isolation of a 35 kDa protein fragment revealed complete identity with polygalacturonase (Petersen et al., 2001). These enzymes degrade cell wall pectin (Swoboda et al., 2004). The group 13 allergens have subsequently been identified in Bahia and Johnson GP, both as 55 kDa proteins with high N-peptide sequence homology to that of Zea m 13 (Campbell et al., 2015; Davies et al., 2011a). Two isoforms of native Sor h 13 with observed molecular weights of 55 and 54 kDa were purified from Johnson GP (Campbell et al., 2015). Whilst eight gene transcripts encoding polygalacturonase were present in the Johnson GP transcriptome, two detected in the proteome corresponded to the purified isoforms designated Sor h 13.0101 and Sor h 13.0201 by mass spectrometry analysis (Campbell et al., 2015).

2.3. Pan-allergens: group 7 and 12

Group 12 GP allergens are profilins, ubiquitous proteins found in all eukaryotic organisms that are pan-allergen responsible for cross reactivity between pollen, latex and plant foods (Matricardi et al., 2016). Profilins are 12–16 kDa actin-binding protein involved in the generation of the cytoskeleton (Gunning et al., 2015). There were three transcripts encoding profilins in Johnson GP that were present in the proteome however, there were no IgE reactive spots consistent with profilin detected on two dimensional immunoblotting with sera pooled from GP-allergic patients from a subtropical region (Campbell et al., 2015). To date, the frequency of IgE reactivity with profilins of subtropical grass pollens has not been established for clinically relevant patient cohorts.

Group 7 GP allergens have been classified as polcalcins, due to the presence of two calcium binding domains. Cyn d 7 was cloned from Bermuda GP and plaque blots of recombinant Cyn d 7 were found to be IgE reactive in 10% of 30 patients from a temperate region of Melbourne. Cyn d 7 showed protein sequence similarity with Bet v 4 (66%) from Birch and Bra r 1 (30%) from oilseed rape, two known calcium binding allergens (Smith et al., 1997; Suphioglu et al., 1997). Four of 68 cDNA transcripts encoding polcalcins were expressed as proteins in the Johnson GP proteome, but IgE reactivity was not detected with pooled GP-allergic sera from a subtropical region (Campbell et al., 2015).

2.4. Minor allergen groups: group 2, 4, 23 and 24

Group 4 GP allergens are metallo-flavoprotein or berberine- bridge orthologues that function as an oxidase in biosynthesis of pollen-specific secondary metabolites (Huang et al., 2012a). Purified Cyn d 4 was IgE reactive in 5 of 10 patients with seasonal allergic rhinitis patients and Bermuda-GP allergy from Florida (Su et al., 1996). One cDNA transcript of the Johnson GP transcriptome matched known GP group 4 allergens, but IgE reactivity was not observed with pooled sera of GP-allergic patients from a subtropical region (Campbell et al., 2015).

Cyn d 24 is exclusively reported in Bermuda GP. This allergen shares homology with pathogenesis-related protein, PR-1, ranging from 45.2 to 49.6% amino acid identity with homologous proteins in maize and barley (*Hordeum vulgare*) (Chow et al., 2005). These are stress proteins produced by plants following bacterial or fungal infections, flooding or freezing temperatures (Stintzi et al., 1993). The frequency of IgE reactivity with Cyn d 24 was 29% of 21 Bermuda GP-allergic asthma patients from Taiwan (Horng-Der et al., 1988).

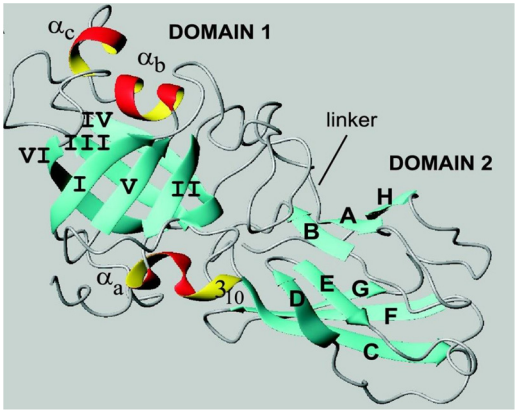

Fig. 1. Ribbon model of Zea m 1 showing the two globular domains and their anti-parrallel beta sheet fold. Adapted from Yennawar et al. 2006 (Yennawar et al., 2006).

The group 2 GP allergens of Johnson GP are 12 kDa proteins with pI of 4.9 and 5.9 for the two isoforms. Whilst being similar to, they are distinct from the group 1 allergen carboxy-terminal domain and clustered separately from group 1 allergen in a dendrogram of GP groups 1, 2 and 3 cDNA transcripts (Campbell et al., 2015). Zea m 2 has been mentioned in several studies, and shares similarity with Zea m 3, but its IgE reactivity has not been evaluated (Oldenburg et al., 2011; Petersen et al., 2006). In studies of the temperate Timothy GP, the corresponding group 2 and 3 allergens, Phl p 2 and Phl p 3, have high IgE cross-reactivity and homology to the C terminal domain of beta-expansins i.e. group 1 allergens (Matricardi et al., 2016; Devanaboyina et al., 2014). Three isoforms of Sor h 2 were identified and found to bind IgE reactivity of GP-allergic sera from patients in subtropical Queensland, Australia (Campbell et al., 2015).

The group 23 GP allergen, Sor h 23 was observed as 29 kDa proteins with isoforms of pI of 5.7 and 6.9. Sor h 23 was found to be larger than its closest homolog, Cyn d 23 (22.2 kDa, 6.22 pI) suggesting they are quite different (Campbell et al., 2015). Whilst Cyn d 23 is listed as an allergen in the WHO/IUIS database, its existence is only discussed (Yang et al., 2005) and there is a lack of studies on its frequency of IgE reactivity with relevant patient sera and its biochemical function.

3. Subtropical grass allergen structures

3.1. Zea m 1

The crystal structure of the β-expansin group 1 pollen allergen Zea m 1 isoform d (Fig. 1), from purified maize pollen, was elucidated by x-ray crystallography to 2.75-A resolution (Yennawar et al., 2006). Termed EXPB1 (Genbank AAO45608), it consists of two globular domains packed closely and has a groove with potential to bind a glycan backbone of approximately 10 sugar residues. The N-terminal domain 1, has sequence similarity of 20% with the catalytic domain of family-45 glycoside hydrolases. The C-terminal domain 2 of Zea m 1, has between 10 to 35% amino acid identity to group 2 and 3 allergens, which are expansin- like proteins (Yennawar et al., 2006).

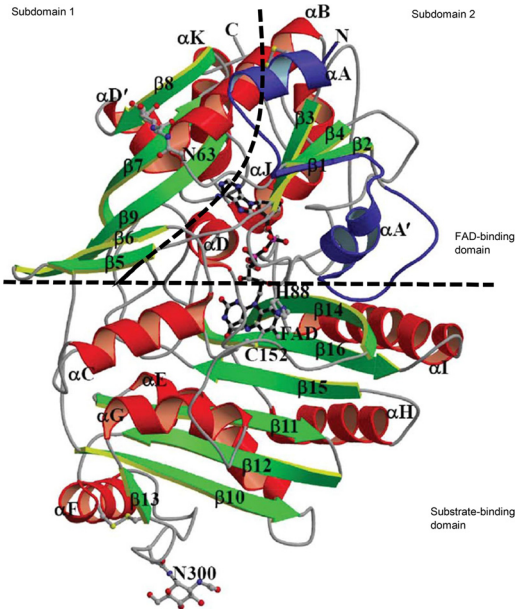

Fig. 2. Stereoview of Cyn d 4 crystal structure displayed as ribbon and stick figure, N-terminal segment in blue. Adapted from Huang et al., 2012a.

3.2. Cyn d 4

The crystal structure of purified natural Cyn d 4 (Fig. 2), was reported at 2.15 A resolution (Huang et al., 2012a). It has structural similarity to the vanillyl alcohol oxidase (VAO) superfamily with two distinct domains; an FAD-binding domain and substrate-binding domain. The FAD-binding domain includes a series of N-terminal and C-terminal residues and folds into two subdomains; one with five β-strands encircled by five α- helices (subdomain 1), and another with 4 core β-strands encompassed within three α- helices (subdomain 2). The substrate binding domain consists of seven β-strands surrounded by five α-helices. Two N-glycosylation sites were also observed. Cyn d 4 also contains an FAD cofactor that is covalently linked to His88 and Cys152, making it a bicovalent flavoprotein. There is also a deep, large cavity in which most of the residues are hydrophobic. Taken together, these features indicate Cyn d 4 has oxidase functions involved in biosynthesis. Cyn d 4 contains a large number of solvent-exposed, positively-charged residues, which result in its basic pI of close to 10. When compared with models of group 4 allergens, two conserved patches were identified as putative antibody or IgE binding regions while five conserved clusters identified could comprise of cross-reactive epitopes (Huang et al., 2012a).

4. Isoallergens and isoforms

Allergens can exist as isoallergens or isoforms with varying molecular weights and isoelectric points (pI) (Pomes et al., this issue). Isoallergens show sequence differences and can arise from separate gene loci, as exemplified by two Zea m 1 isoforms (GenBank accession nos. AY104999 and AY104125) having higher sequence similarity with Lol p 1 and Phl p 1 than with two other Zea m 1 isoforms (AY197352 and AY197353) (Li et al., 2003). While all four Zea m 1 isoforms exhibited similar biochemical activity (cell wall extension), there were differences in protein abundance and reactivity with monoclonal antibodies (Li et al., 2003). This implies structural and potentially, immunological differences between isoforms. Table A2 summarizes the known isoforms of subtropical GP allergens.

The number of isoforms and isoallergens of subtropical grasses listed in Table A.2 were compiled from the WHO/IUIS Allergen Nomenclature database. Cyn d 1 has two identified isoallergens; Cyn d 1.01 with 7 variants and Cyn d 1.02 with 4 variants. Cyn d 1 was first described as a 32 kDa protein with a pI of 6.2. Chang et al were the first to identify between four to ten isoforms with pI ranging from 5.6 to 7.3, classified by acidity (acidic, basic, neutral) and with the basic and neutral isoforms having lower molecular weight (Chang et al., 1995). More importantly, the basic and neutral isoforms were found to have higher RAST inhibition compared to the acidic isoforms for inhibition of pooled patient IgE reactivity with the basic isoform fraction, suggesting unique allergic determinants in the basic and neutral isoforms. However, the acidic isoform fraction showed higher level of IgE reactivity with pooled serum than the neutral isoform fraction.

Screening of the Bermuda cDNA library for unidentified Cyn d 1 isoallergens revealed four new cDNA clones encoding acidic proteins that share 86% identity with the basic isoforms identified by Chang et al (Au et al., 2002; Chang et al., 1995). Further comparisons of these two groups of Cyn d 1 isoallergens revealed different net charges for the acidic isoallergen and for the basic isoallergen, different signal peptides of 18 amino acid residues for acidic and 26 for basic, as well as the presence of additional GA residues at the C-terminus in some basic isoallergens that are not present in the acidic isoallergens.

Two isoforms of Sor h 1 were discovered separately; one basic Sor h 1.02 and acidic Sor h 1.01, but their IgE reactivity has not been directly compared (Avjioglu et al., 1993; Campbell et al., 2015).

Currently, there is a lack of information regarding the clinical implications of multiple isoallergens from subtropical GP for instance isoforms of Cyn d 1, Zea m 1 and Sor h 1. However, studies on other inhalant allergens have demonstrated immunological differences between their respective isoallergens (Christensen et al., 2010; Hartl et al., 1999). Further research on immunoreactivity of subtropical GP isoallergens using quantitative methods with relevant, clinically-affected patients is needed to improve our understanding of the importance of particular isoforms for diagnositic accuracy or therapeutic benefit.

5. Diagnostics

Clinical utilization of component-resolved diagnostics (CRD) complements skin prick tests and serum-specific IgE testing with whole extracts (Scala et al., 2010). When compared to skin prick tests, CRD is more specific for identifying the primary sensitizing allergen or disease-causing allergen source, which is essential for precise selection of immunotherapy (Gonzalez-Mancebo et al., 2017; Saltabayeva et al., 2017).

nCyn d 1 was the first subtropical GP allergen to be commercially available for CRD, specifically in the ImmunoCAP system (Thermofisher, Sweden) (Matthiesen et al., 1991). The utility of this assay system was recently demonstrated by a cross-sectional study in southern China that used CRD to investigate sensitization profiles of purified allergens from Bermuda, Timothy and *Humulus scandens* in 346 patients with allergic rhinitis and/or asthma (Luo et al., 2017; Luo et al., 2016). nCyn d 1 has also been included as one of the 112 allergens on the ImmunoCAP ISAC multiplex array (ThermoFisher, 2012). rCyn d 12 was also tested as part of the ISAC array in the first extensive cross-sectional study of 23, 077 Italian patients, but it is not commercially available (Scala et al., 2010).

An issue is that nCyn d 1 is glycosylated near the amino terminus, thus testing of IgE reactivity with nCyn d 1 in populations not exposed

to Bermuda grass can give positive results that are difficult to reconcile clinically (Cabauatan et al., 2014). These positive reactions could be low affinity cross-reactivity of IgE primarily specific to temperate GP allergens, or due to non- specific, clinically-irrelevant binding to the cross-reactive carbohydrate component. Note that in the Philippines, a tropical region with a low population frequency of reactivity with GP (Davies, 2014), there was no difference in IgE reactivity to nCyn d 1 in patients with symptomatic allergic rhinitis and those reporting no allergic rhinitis, and the IgE binding to nCyn d 1 could be reduced by inhibition with Cross-reactive carbohydrate determinant (CCD)-containing nPhl p4 or by deglycosylation (Cabauatan et al., 2014).

In Australia where GP allergy frequency is high, specific IgE reactivity was detected with recombinant Pas n 1 (Davies et al., 2008), indicating that the carbohydrate component is not involved in IgE binding to this rPas n 1. Moreover, in separate studies in Florida USA, deglycosylation of the group 1 allergen of Bahia GP did not diminish IgE binding (Ghobrial et al., 2002), indicating that in geographically relevant locations CCD is not the reason for detection of IgE binding with group 1 allergens of subtropical grass pollens.

The potential for nPas n 1 in the diagnosis of patients with allergic rhinitis due to Bahia GP allergy was shown with biotinylated nPas n 1 coated onto a streptavidin ImmunoCAP (Timbrell et al., 2014). Serum specific IgE towards nPas n 1 of 182 GP-allergic patients, with clinical history of allergic rhinitis, was highly correlated with BaGP SPT ($r = 0.795$) and BaGP IgE ($r = 0.951$). Furthermore, this assay showed high sensitivity detecting of Pas n 1 specific IgE (92.4%, cut-off at 0.225 kU/L), high specificity (93.1%) and low inter-assay co- efficient of variation (6.92%) (Timbrell et al., 2014). However, it was also observed that 8% of other allergy patients also showed IgE reactivity with natural Pas n 1 suggesting that some positive responses in this assay format could be due to cross-reactive carbohydrate moieties (Timbrell et al., 2014), indicating the need for recombinant subtropical grass pollen group 1 allergen components for diagnosis.

6. Treatment

Allergen immunotherapy (AIT) is a commonly administered and effective treatment for controlling allergic rhinitis due to GP (Walker et al., 1995; Bousquet et al., 1998). While subcutaneous and sublingual immunotherapy options are safe and effective, for reducing clinically symptoms and medication use in adults and children with allergic rhinitis and asthma (Dhami et al., 2017a,b), they both require repeated doses to achieve long-term efficacy and have rare but potentially harmful side effects (Klimek et al., 2016).

New treatment methods are being trialled including synthetic peptide immuno-regulatory epitopes (SPIRE) (Larché and Kay, 2004). This treatment method requires lower doses and a shorter treatment course compared to current SCIT or SLIT and due to its short length, has lowered chance to cross-link IgE on mast cells and basophils (Creticos, 2014). SPIRE for GP allergy is being developed, comprising of T-cell epitope peptides from the subtropical Cyn d 1 and a four temperate GP group 5 allergens; Lol p 5 (Ryegrass), Phl p 5 (Timothy), Dac g 5 (orchard) and Hol l 5 (velvet). In a multicentre phase II clinical trial involving 282 patients with a randomized, double-blind, placebo-controlled design, following 14 weeks of treatment with this GP SPIRE significantly reduced rhinoconjunctivitis symptoms in an Environmental Exposure Unit, and exposure to a natural pollen season in Canada (Ellis et al., 2017). An optional follow-up study on this cohort after a second natural pollen season showed a sustained treatment effect after cessation of dosing (Ellis et al., 2015).

A DNA vaccine candidate for subtropical GP has been designed by inserting cDNA of Cyn d 1 into the vector pcDNA3 (Huang et al., 2012b). A model of BALB/C mice sensitized by intraperitoneal injection twice to rCyn d 1, was immunized by intramuscular injection of recombinant plasmid Cyn d 1 (pCyn d 1). This induced Th1 responses, characterized by increased allergen specific IgG and interferon- γ levels from CD4 + and CD8 + as well as suppression of specific-IgE in serum (Huang et al., 2012b). A similar study on neonatal BALB/C mice vaccinated with pCyn d 1 and sensitized twice with intraperitoneal rCyn d 1 showed a similar suppression of specific IgE response, induction of Th1 response and IL-4 secretion in spleen (Huang et al., 2014).

7. Summary and conclusions

The differences in frequency of SPT, levels of IgE reactivity and patterns of cross-reactivity reported for subtropical grass pollen allergens of Bahia, Bermuda and Johnson GP are likely to be clinically relevant for patients in subtropical regions. Others report the ratio of allergen specific IgE to total IgE (Di Lorenzo et al., 2009), the number allergen components recognized within Timothy GP (Darsow et al., 2014), and the recognition of particular allergen component (Savi et al., 2013), to indicate severity or progression of allergic respiratory disease (Hatzler et al., 2012). With increasing capacity to measure levels of specific IgE to allergen components, implementation of more quantitative and molecularly defined allergy testing is likely to be clinically helpful for subtropical GP allergen components. Further research with quantitative methods is needed to determine the rates and levels of sensitisation to standardized recombinant allergen components such as Cyn d 1, Pas n 1, Sor h 1, Cyn d 4, Pas n 13 and Sor h 13 in relevant patients with allergic respiratory diseases from subtropical regions to evaluate the importance of particular subtropical GP allergen component isoforms and to drive the advancement of more defined and specific diagnosis and treatment options for subtropical GP allergy.

Conflict of interest statement

JMD leads the NHMRC AusPollen Partnership Project (GNT 1116107) with matching cash and in kind co-sponsorship from The Australasian Society for Clinical Immunology and Allergy, Asthma Australia, Bureau of Meteorology, Commonwealth Scientific and Industrial Research Organisation, Stallergenes Australia, Meteorology Switzerland. She is an investigator of the ARC Discovery (DP170101630), undertakes research for the Bureau of Meteorology on a Thunderstorm Asthma Pollen Forecasting Project. JMD received funds from The Victorian State government of Australia Department of Health and Human Services to report on a review of literature and public health implications of thunderstorm asthma and from the Bureau of Meteorology for implementation of the Victorian Thunderstorm Asthma Pollen Surveillance (VicTAPS) project. She has received grants from the NHMRC, National Foundation for Medical Research Innovation, the Allergy and Immunology Foundation of Australasia, Asthma Australia, Queensland University of Technology, Asthma Australia, The University of Queensland and a contracted research grants from Stallergenes (France), Abionic (Switzerland), in-kind provision of materials from ThermoFisher (Sweden) and services from Sullivan Nicolaides Pathology (QLD, Australia). She is an inventor on patents assigned to QUT granted in Australia, allowed in USA and three applications pending examination in Australia, USA and Europe. Assoc. Prof. Davies's institute has received Honorarium payments and travel expenses for education sessions and conference presentations from Stallergenes Australia, GlaxoSmithKliene, Wymedical, and Meda Pharmaceuticals.

Acknowledgements

TK receives an International Post Graduate Research Award Scholarship with an Excellence Top Up Scholarship from Queensland University of Technology. JMD receives grant funding for related research from the National Health and Medical Research Council of Australia, Australian Research Council, the National Health and Medical Research Foundation and Queensland University of Technology.

Appendix A

Table A1
Biochemical families, biological function crystal structure and post-translational modifications of known subtropical grass pollen allergens.

Biochemical family	Biological function	Protein structure available	Name	Post-translational modifications	References
β-expansin (Group 1); 29-31 kDa	Cell-wall loosening to allow cell enlargement during plant growth (Wang et al., 2016), (Cosgrove et al., 1997).	Zea m 1	Pas n 1 Cyn d 1 Sor h 1 Zea m 1	N-glycosylation	Davies et al. (2008), Matthiesen et al. (1991), Avjioglu et al. (1993), Campbell et al. (2015) , Li et al. (2003)
Expansin-like protein/C-terminal β-expansin (Group 2); 12 kDa	Similar to β-expansin	N/A	Sor h 2		Campbell et al. (2015)
Metallo- flavoprotein/ glycoprotein (Group 4); 60 kDa, pI 9.3	Berberine bridge enzyme orthologue (Davies, 2014) Oxidase involved in biosynthesis of pollen-specific apolar secondary metabolite	Cyn d 4	Cyn d 4		Huang et al. (2012a)
Polcalcin (Group 7); 12 kDa	Two calcium-binding domains (EF-hands)	Cyn d 7 (Smith et al., 1997)	Cyn d 7		Suphioglu et al. (1997)
Profilin (Group 12); 14.1 kDa	Actin-binding protein	N/A	Cyn d 12 Zea m 12		Asturias et al. (1997) Kovar et al. (2000)
Polygalacturonase (Group 13); 54-55 kDa	Pectin-degrading enzymes (Swoboda et al., 2004)	N/A	Pas n 13 Sor h 13 Zea m 13	N-glycosylation	Davies et al. (2011b) Campbell et al. (2015) Petersen et al. (2006)
Pathogenesis-related protein (Group 24); 21 kDa	Proteins released by plants when faced with environmental stress (Stintzi et al., 1993)	N/A	Cyn d 24	N-glycosylation	Chow et al. (2005), Stintzi et al. (1993)

Table A2
Subtropical grass pollen allergens purification method, recombinant expression systems, variants and protein/nucleotide accession numbers.

Allergen	Frequency of IgE reactivity	Natural allergens (purification methods)	Recombinant allergens (expression system)	Isoallergen / variants (IUIS nomenclature)	Accession numbers (allergen.org)			References
					Genbank Nucleotide	Genbank Protein	Uniprot	
Pas n 1	85% of 55 to 92% of 182 GP-allergic patients	nPas n 1 - ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography	rPas n 1– expressed in *E. coli* transformed with pET-28a-Pas n 1 vector.	Pas n 1.0101	EU327342	ACA23876	B8PYF3	Ghobrial et al. (2002), Drew et al. (2011), Davies et al. (2008), Davies et al. (2005)
Cyn d 1	76% of 21 GP-allergic patients, 100% in 44 Bermuda GP SPT positive patients	nCyn d 1	rCyn d 1 - vector pTrc 99A expressed in *E. coli* and yeast expression vector pHIL-S1 expressed in *Pichia pastoris*	Cyn d 1.0101	S83343	AAB50734	O04701	Smith et al. (1996), Shen et al. (1988), Ford and Baldo (1987), Matthiesen et al. (1991)
		-concanavalin A- Sepharose affinity chromatography, and carboxymethyl-Sepharose chromatography		Cyn d 1.0102	N/A	N/A	N/A	Chang et al. (1999)
				Cyn d 1.0103	N/A	N/A	N/A	
				Cyn d 1.0104	N/A	N/A	N/A	
				Cyn d 1.0105	N/A	N/A	N/A	
				Cyn d 1.0106	N/A	N/A	N/A	
				Cyn d 1.0107	N/A	N/A	N/A	
				Cyn d 1.0201	AF177030	AAK96255	Q947S7	
				Cyn d 1.0202	AF177378	AAL14077	Q947S6	
				Cyn d 1.0203	AF177380	AAL14079	Q947S4	
				Cyn d 1.0204	AF159703	AAF80379	Q9FVM0	
Sor h 1	76% of 64 GP-allergic patients	nSor h 1 – ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography		Sor h 1.0101	KF887425	AIL01316	C5WMS3	Avjioglu et al. (1993), Campbell et al. (2015)
				Sor h 1.0201	KF887426	AIL01317	A0A077B4J2	
Zea m 1	Positive for maize pollen exposed individuals	nZea m 1 – acetate buffer extraction, Carboxymethyl-sepharose chromatography, CM-silica HPLC		Zea m 1.0101	L14271	AAA33496	Q07154	Wu et al. (2001) Petersen et al. (2006)
				N/A	N/A	AY104999	N/A	Li et al. (2003) these four not named in IUIS
				N/A	N/A	AY104125	N/A	*designated EXPB9, gene distinct form EXPB1
				N/A	N/A	AY197352*	N/A	**identical sequence to Zea m 1.0101 (EXPB1) but longer
				N/A	N/A	AY197353**	N/A	
Sor h 2	IgE reactive by immunoblotting in Johnson GP positive patient	nSor h 2 – ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography	rSor h 2 expressed in *E.coli* transformed with pET-28a plasmid. (Davies, unpublished)	Sor h 2.0101	KF887427	AIL01318	A0A077B7S9	Campbell et al. (2015)
				Sor h 2.0201	KF887428	AIL01319	A0A077B2S0	
Cyn d 4	5 of 10 patients with seasonal allergic rhinitis	nCyn d 4 – ammonium sulphate precipitation, Sephadex G-25, ion-exchange, blue gel affinity, and reverse-phase high- performance liquid chromatography		N/A	AY451241	AAS02108	Q5QJ60	Su et al. (1996) Huang et al. (2012b)
Cyn d 7	10% of 30 GP-allergic patients with AR by IgE immunoblotting		rCyn d 7 – λgtll cDNA library of mature Bermuda anther screened for IgE binding of pooled GP-allergic sera	Cyn d 7.0101	X91256	CAA62634	P94092	Suphioglu et al. (1997)

(*continued on next page*)

Table A2 (*continued*)

Allergen	Frequency of IgE reactivity	Natural allergens (purification methods)	Recombinant allergens (expression system)	Isoallergen / variants (IUIS nomenclature)	Accession numbers (allergen.org)			References
					Genbank Nucleotide	Genbank Protein	Uniprot	
Cyn d 12	20% in 30 GP-allergic patients		rCyn d 12– *E. coli* BL21 (DE3) using pKN172 vector	Cyn d 12.0101	Y08390	CAA69670	O04725	Asturias et al. (1997), Kao et al. (2005)
Zea m 12			rZea m 12 – vector pET-23a expressed in *E. coli* strain BL21(DE3)	Zea m 12.0101 Zea m 12.0102 Zea m 12.0103 Zea m 12.0104 Zea m 12.0105	X73279 X73280 X73281 AF032370 AF201459	CAA51718 CAA51719 CAA51720 AAB86960 AAG35601	P35081 P35082 P35083 O22655 Q9FR39	Kovar et al. (2000)
Pas n 13	48% of 71 GP-allergic patients	nPas n 13 -ammonium sulphate precipitation, hydrophobic interaction chromatography and size exclusion chromatography		N/A	N/A	N/A	N/A	Davies et al. (2011b)
Sor h 13	43.8% of 64 GP-allergic patients	nSor h 13 – ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography		Sor h 13.0101 Sor h 13.0201	KF887429 KF887430	AIL01320 AIL01321	A0A077B155 A0A077B569	Campbell et al. (2015)
Zea m 13	IgE reactive by immunoblotting in patients with maize pollen exposure		rZea m 13 – cDNA cloned into λ-ZapXR bacteriophage, plaque-purified	N/A	N/A	N/A	Q1ZYQ5	Petersen et al. (2006)
Cyn d 24	29% of 21 asthmatic, Bermuda GP-allergic patients	nCyn d 24 – CM-TSK column and reverse HPLC		Cyn d 24.0101	AY720896	AAU15051	Q647J6	Chow et al. (2005), Horng-Der et al. (1988)

AR; allergic rhinitis, GP; grass pollen, ELISA; Enzyme linked immunosorbent assay; n, natural; r, recombinant.

References

Aalberse, R.C., 2007. Assessment of allergen cross-reactivity. Clin. Mol. Allergy 5, 2 doi: 1476-7961-5-2 [pii] 10.1186/1476-7961-5-2.

Andersson, K., Lidholm, J., 2003. Characteristics and immunobiology of grass pollen allergens. Int. Arch. Allergy Immunol. 130 (2), 87–107. http://dx.doi.org/10.1159/000069013.

Asturias, J.A., Arilla, M.C., Gomez- Bayon, N., Martinez, J., Martinez, A., Palacios, R., 1997. Cloning and high level expression of Cynodon dactylon (Bermuda grass) pollen profilin (Cyn d 12) in Escherichia coli: purification and characterization of the allergen. Clin. Exp. Allergy 27 (11), 1307–1313 Retrieved from.

Au, L.C., Lin, S.T., Peng, H.J., Liang, C.C., Lee, S.S., Liao, C.D., Chang, Z.N., 2002. Molecular cloning and sequence analysis of full-length cDNAs encoding new group of Cyn d 1 isoallergens. Allergy 57 (3), 215–220.

Avjioglu, A., Creaney, J., Smith, P.M., Taylor, P., Singh, M.B., Knox, R.B., 1993. Cloning and characterization of the major allergen of sorghum halepense, a subtropical grass. Mol. Biol. Immunol. Allerg. 161–164.

Beggs, P.J., 2009. Climate change and plant food allergens. J. Allergy Clin. Immunol. 123 (1), 271–272 author reply 272. doi: S0091-6749(08)01871-X [pii] 10.1016/j.jaci.2008.10.025.

Bousquet, J., Lockey, R., Malling, H.-J., 1998. Allergen immunotherapy: therapeutic vaccines for allergic diseases A WHO position paper. J. Allergy Clin. Immunol. 102 (4), 558–562. http://dx.doi.org/10.1016/S0091-6749(98)70271-4.

Burton, M.D., Papalia, L., Eusebius, N.P., O'Hehir, R.E., Rolland, J.M., 2002. Characterization of the human T cell response to rye grass pollen allergens Lol p 1 and Lol p 5. Allergy 57 (12), 1136–1144.

Cabauatan, C.R., Lupinek, C., Scheiblhofer, S., Weiss, R., Focke-Tejkl, M., Bhalla, P.L., ... Valenta, R., 2014. Allergen microarray detects high prevalence of asymptomatic IgE sensitizations to tropical pollen-derived carbohydrates. J. Allergy Clin. Immunol. 133 (3), 910–914. http://dx.doi.org/10.1016/j.jaci.2013.10.004. e915.

Calabria, C.W., Dice, J., 2007. Aeroallergen sensitization rates in military children with rhinitis symptoms. Ann. Allergy Asthma Immunol. 99 (2), 161–169. http://dx.doi.org/10.1016/S1081-1206(10)60640- 0.

Campbell, B.C., Gilding, E.K., Timbrell, V., Guru, P., Loo, D., Zennaro, D., ... Davies, J.M., 2015. Total transcriptome, proteome, and allergome of johnson grass pollen, which is important for allergic rhinitis in subtropical regions. J. Allergy Clin. Immunol. 135 (1), 133–142. http://dx.doi.org/10.1016/j.jaci.2014.06.034.

Chang, Z.-N., Liu, C.-C., Tam, M.F., Peng, H.-J., Tsai, J.-J., Han, S.-H., 1995. Characterization of the isoforms of the group I allergen of *Cynodon dactylon*. J. Allergy Clin. Immunol. 95 (6), 1206–1214. http://dx.doi.org/10.1016/S0091-6749(95)70077- 3.

Chang, Z.N., Peng, H.J., Lee, W.C., Chen, T.S., Chua, K.Y., Tsai, L.C., ... Han, S.H., 1999. Sequence polymorphism of the group 1 allergen of Bermuda grass pollen. Clin. Exp. Allergy 29 (4), 488–496 Retrieved from.

Chow, L.P., Chiu, L.L., Khoo, K.H., Peng, H.J., Yang, S.Y., Huang, S.W., Su, S.N., 2005. Purification and structural analysis of the novel glycoprotein allergen Cyn d 24, a pathogenesis-related protein PR-1, from Bermuda grass pollen. FEBS J. 272 (24), 6218–6227. http://dx.doi.org/10.1111/j.1742- 4658.2005.05000.x.

Christensen, L.H., Riise, E., Bang, L., Zhang, C., Lund, K., 2010. Isoallergen variations contribute to the overall complexity of effector cell degranulation: effect mediated through differentiated IgE affinity. J. Immunol. 184 (9), 4966–4972. http://dx.doi.org/10.4049/jimmunol.0904038.

Cohen, S.G., Zelaya-Quesada, M., 2002. Portier, Richet, and the discovery of anaphylaxis: a centennial. J. Allergy Clin. Immunol. 110 (2), 331–336.

Cosgrove, D.J., Bedinger, P., Durachko, D.M., 1997. Group I allergens of grass pollen as cell wall-loosening agents. Proc. Natl. Acad. Sci. 94 (12), 6559–6564. Retrieved from http://www.pnas.org/content/94/12/6559.a bstract.

Creticos, P.S., 2014. Advances in synthetic peptide immuno-regulatory epitopes. World Allergy Organ. J. 7 (1), 30.

Darsow, U., Brockow, K., Pfab, F., Jakob, T., Petersson, C.J., Borres, M.P., ... Huss-Marp, J., 2014. Allergens. heterogeneity of molecular sensitization profiles in grass pollen allergy–implications for immunotherapy? Clin. Exp. Allergy 44 (5), 778–786. http://dx.doi.org/10.1111/cea.12303.

Davies, J.M., Bright, M.L., Rolland, J.M., O'Hehir, R.E., 2005. Bahia grass pollen specific IgE is common in seasonal rhinitis patients but has limited cross-reactivity with Ryegrass. Allergy 60 (2), 251–255 Retrieved from.

Davies, J.M., Mittag, D., Dang, T.D., Symons, K., Voskamp, A., Rolland, J.M., O'Hehir, R.E., 2008. Molecular cloning, expression and immunological characterisation of pas n 1, the major allergen of Bahia grass *Paspalum notatum* pollen. Mol. Immunol. 46 (2), 286–293. http://dx.doi.org/10.1016/j.molimm.2008.08.267.

Davies, J.M., Dang, T.D., Voskamp, A., Drew, A.C., Biondo, M., Phung, M., ... O'Hehir, R.E., 2011a. Functional immunoglobulin E cross-reactivity between Pas n 1 of Bahia grass pollen and other group 1 grass pollen allergens. Clin. Exp. Allergy 41 (2), 281–291. http://dx.doi.org/10.1111/j.1365-2222.2010.03670.x.

Davies, J.M., Voskamp, A., Dang, T.D., Pettit, B., Loo, D., Petersen, A., ... O'Hehir, R.E., 2011b. The dominant 55 kDa allergen of the subtropical Bahia grass (*Paspalum notatum*) pollen is a group 13 pollen allergen, Pas n 13. Mol. Immunol. 48 (6-7), 931–940. http://dx.doi.org/10.1016/j.molimm.2010.12.013.

Davies, J.M., Li, H., Green, M., Towers, M., Upham, J.W., 2012. Subtropical grass pollen allergens are important for allergic respiratory diseases in subtropical regions. Clin. Transl. Allergy 2 (1), 4. http://dx.doi.org/10.1186/2045-7022-2-4.

Davies, J.M., 2014. Grass pollen allergens globally: the contribution of subtropical grasses to burden of allergic respiratory diseases. Clin. Exp. Allergy 44 (6), 790–801. http://dx.doi.org/10.1111/cea.12317.

Devanaboyina, S.C., Cornelius, C., Lupinek, C., Fauland, K., Dall'Antonia, F., Nandy, A., ... Keller, W., 2014. High-resolution crystal structure and IgE recognition of the major grass pollen allergen Phl p 3. Allergy 69 (12), 1617–1628. http://dx.doi.org/10.1111/all.12511.

Dhami, S., Kakourou, A., Asamoah, F., Agache, I., Lau, S., Jutel, M., ... Sheikh, A., 2017a. Allergen immunotherapy for allergic asthma: a systematic review and meta-analysis. Allergy 72, 1825–1848. http://dx.doi.org/10.1111/all.13208.

Dhami, S., Nurmatov, U., Arasi, S., Khan, T., Asaria, M., Zaman, H., ... Sheikh, A., 2017b. Allergen immunotherapy for allergic rhinoconjunctivitis: a systematic review and meta-analysis. Allergy 72, 1597–1631. http://dx.doi.org/10.1111/all.13201.

Di Lorenzo, G., Mansueto, P., Pacor, M.L., Rizzo, M., Castello, F., Martinelli, N., ... Ditto, A.M., 2009. Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. J. Allergy Clin. Immunol. 123 (5), 1103–1110. http://dx.doi.org/10.1016/j.jaci.2009.02.012. e1101-1104, [Research Support, Non-U.S. Gov't].

Drew, A.C., Davies, J.M., Dang, T.D., Rolland, J.M., O'Hehir, R.E., 2011. Purification of the major group 1 allergen from Bahia grass pollen, Pas n 1. Int. Arch Allergy Immunol. 154 (4), 295–298. http://dx.doi.org/10.1159/000321821.

Ellis, A., Frankish, C.W., Armstrong, K., Larché, M., Steacy, L., Hafner, R., O'Hehir, R., 2015. Persistent treatment effect with grass synthetic peptide immuno-regulatory epitopes in grass allergy symptoms in an environmental exposure unit challenge after a second season of natural pollen exposure. J. Allergy Clin. Immunol. 135 (2). http://dx.doi.org/10.1016/j.jaci.2014.12.1457. p. AB158.

Ellis, A.K., Frankish, C.W., O'Hehir, R.E., Armstrong, K., Steacy, L., Larché, M., Hafner, R.P., 2017. Treatment with grass allergen peptides improves symptoms of grass pollen-induced allergic rhinoconjunctivitis. J. Allergy Clin. Immunol. 140 (2), 486–496. http://dx.doi.org/10.1016/j.jaci.2016.11.043.

Esch, R.E., 2004. Grass pollen allergens. Clin. Allergy Immunol. 18, 185–205. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/15042916.

Etto, T., de, B.C., Prickett, S., Gardner, L.M., Voskamp, A., Davies, J.M., ... Rolland, J.M., 2012. Unique and cross-reactive T cell epitope peptides of the major Bahia grass pollen allergen, Pas n 1. Int. Arch. Allergy Immunol. 159 (4), 355–366. http://dx.doi.org/10.1159/000338290.

Eusebius, N.P., Papalia, L., Suphioglu, C., McLellan, S.C., Varney, M., Rolland, J.M., O'Hehir, R.E., 2002. Oligoclonal analysis of the atopic T cell response to the group 1 allergen of Cynodon dactylon (bermuda grass) pollen: pre- and post-allergen-specific immunotherapy. Int. Arch Allergy Immunol. 127 (3), 234–244. Retrieved from. http://www.online.karger.com/library/karger/renderer/dataset.exe? jcode=IAA&action=render&rendertype=fulltext&uid=IAA.iaa27234.

Ford, S.A., Baldo, B.A., 1987. Identification of Bermuda grass (*Cynodon dactylon*)–pollen allergens by electroblotting. J. Allergy Clin. Immunol. 79 (5), 711–720.

Ghobrial, G., Naser, S.A., Sweeney, M., White, R., 2002. Identification and characterization of the allergenic proteins of Bahia grass (Paspalum notatum) pollen. Int. Arch. Allergy Immunol. 128 (4), 304–309.

Gonzalez-Mancebo, E., Dominguez-Ortega, Blanco-Bermejo, S., Gonzalez-Seco, E., Trujillo, M.J., de la Torre, F., 2017. Comparison of two diagnostic techniques, skin-prick test and component resolved diagnosis in the follow-up of a cohort of paediatric patients with pollinosis. Multicentre pilot study in a highly exposed allergenic area. Allergol. Immunopathol. (Madr.) 45 (2), 121–126. http://dx.doi.org/10.1016/j.aller.2016.04.005.

Gunning, P.W., Ghoshdastider, U., Whitaker, S., Popp, D., Robinson, R.C., 2015. The evolution of compositionally and functionally distinct actin filaments. J. Cell. Sci. 128 (11), 2009–2019.

Gupta, A., 2002. Geoindicators for tropical urbanization. Environ. Geol. 42, 736–742.

Hartl, A., Kiesslich, J., Weiss, R., Bernhaupt, A., Mostbock, S., Scheiblhofer, S., ... Thalhamer, J., 1999. Isoforms of the major allergen of birch pollen induce different immune responses after genetic immunization. Int. Arch. Allergy Immunol. 120 (1), 17–29. http://dx.doi.org/10.1159/000024216.

Hatzler, L., Panetta, V., Lau, S., Wagner, P., Bergmann, R.L., Illi, S., ... Matricardi, P.M., 2012. Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. J. Allergy Clin. Immunol. 130 (4). http://dx.doi.org/10.1016/j.jaci.2012.05.053. 894-901 e895.

Hensel Jr, A.E., Griffith, R.C., 1972. Clinical experiences with *Paspalum notatum* (Bahia grass): a new grass antigen. S. Med. J. 65 (6), 690–693.

Horng-Der, S., Wang, S.R., Tang, R.B., Chang, Z.-N., Su, S.N., Han, S.H., 1988. Identification of allergens and antigens of Bermuda grass (*Cynodon dactylon*) pollen by immunoblot analysis. Clin. Allergy 18 (4), 401–409.

Huang, T.H., Peng, H.J., Su, S.N., Liaw, S.H., 2012a. Various cross-reactivity of the grass pollen group 4 allergens: crystallographic study of the Bermuda grass isoallergen Cyn d 4. Acta Crystallogr. D Biol. Crystallogr. 68 (Pt 10), 1303–1310. http://dx.doi.org/10.1107/s0907444912027552.

Huang, C.F., Chu, C.H., Wu, C.C., Chang, Z.N., Chue, F.L., Peng, H.J., 2012b. Induction of specific Th1 responses and suppression of IgE antibody formation by vaccination with plasmid DNA encoding Cyn d 1. Int. Arch. Allergy Immunol. 158 (2), 142–150. http://dx.doi.org/10.1159/000331140.

Huang, C.F., Wu, C.C., Chu, D.M., Chang, Z.N., Chue, F.L., Peng, H.J., 2014. Neonatal vaccination with plasmid DNA encoding Cyn d 1 effectively prevents allergic responses in mice. Am. J. Rhinol. Allergy 28 (3), e144–147. http://dx.doi.org/10.2500/ajra.2014.28.4020.

Johansen, N., Weber, R.W., Ipsen, H., Barber, D., Broge, L., Hejl, C., 2009. Extensive IgE cross-reactivity towards the Pooideae grasses substantiated for a large number of grass-pollen-sensitized subjects. Int. Arch. Allergy Immunol. 150 (4), 325–334 doi: 000226233 [pii] 10.1159/000226233.

Kao, S.H., Su, S.N., Huang, S.W., Tsai, J.J., Chow, L.P., 2005. Sub-proteome analysis of novel IgE-binding proteins from Bermuda grass pollen. Proteomics 5 (14),

3805–3813.
Klimek, L., Pfaar, O., Worm, M., 2016. New opportunities for allergen immunotherapy using synthetic peptide immuno-regulatory epitopes (SPIREs). Expert Rev. Clin. Immunol. 12 (10), 1123–1135. http://dx.doi.org/10.1080/1744666x.2016.1189825.
Kovar, D.R., Drobak, B.K., Staiger, C.J., 2000. Maize profilin isoforms are functionally distinct. Plant cell 12 (4), 583–598 Retrieved from.
Larché, M., Kay, A.B., 2004. The effects of T cell peptides in patients sensitive to cats. Clin. Exp. Allergy Rev. 252–257. http://dx.doi.org/10.1111/j.1472-9725.2004.00062.x.
Leiferman, K.M., Gleich, G.J., 1976. The cross-reactivity of IgE antibodies with pollen allergens. I. Analyses of various species of grass pollens. J. Allergy Clin. Immunol. 58 (1 PT. 2), 129–139.
Li, L.C., Bedinger, P.A., Volk, C., Jones, A.D., Cosgrove, D.J., 2003. Purification and characterization of four beta-expansins (Zea m 1 isoforms) from maize pollen. Plant. Physiol. 132 (4), 2073–2085.
Luo, W., Huang, H., Zheng, P., Wei, N., Luo, J., Sun, B., Zeng, G., 2016. Major grass pollen allergens and components detected in a Southern Chinese cohort of patients with allergic rhinitis and/or asthma. Mol. Immunol. 78, 105–112. http://dx.doi.org/10.1016/j.molimm.2016.08.013.
Luo, W., Pan, G., Huang, H., Zheng, P., Wei, N., Zhang, Y., ... Sun, B., 2017. A component-resolved diagnostic approach for a study on grass pollen allergens in Chinese southerners with allergic rhinitis and/or asthma. J. Vis. Exp. 124. http://dx.doi.org/10.3791/55723.
Martin, B.G., Mansfield, L.E., Nelson, H.S., 1985. Cross-allergenicity among the grasses. Ann. Allergy 54 (2), 99–104. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi? cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3970392.
Matricardi, P.M., Kleine- Tebbe, J., Hoffmann, H.J., Valenta, R., Hilger, C., Hofmaier, S., ... Ollert, M., 2016. EAACI molecular allergology user's guide. Pediatr Allergy Immunol. 27 (Suppl. 23), 1–250. http://dx.doi.org/10.1111/pai.12563.
Matthiesen, F., Schumacher, M.J., Lowenstein, H., 1991. Characterization of the major allergen of Cynodon dactylon (Bermuda grass) pollen, Cyn d 1. J. Allergy Clin. Immunol. 88 (5), 763–774.
Morgan, J.A., LeCain, D.R., Pendall, E., Blumenthal, D.M., Kimball, B.A., Carrillo, Y., ... West, M., 2011. C4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi- arid grassland. Nature 476 (7359), 202–205 doi: nature10274 [pii] 10.1038/nature10274.
Nony, E., Timbrell, V., Hrabina, M., Boutron, M., Solley, G., Moingeon, P., Davies, J.M., 2015. Specific IgE recognition of pollen allergens from subtropic grasses in patients from the subtropics. Ann. Allergy Asthma Immunol. 114 (3), 214–220. http://dx.doi.org/10.1016/j.anai.2014.12.005. e212.
Oldenburg, M., Petersen, A., Baur, X., 2011. Maize pollen is an important allergen in occupationally exposed workers. J. Occup. Med. Toxicol. (Lond. Engl.) 6, 32. http://dx.doi.org/10.1186/1745-6673-6-32.
Petersen, A., Suck, R., Hagen, S., Cromwell, O., Fiebig, H., Becker, W.M., 2001. Group 13 grass allergens: structural variability between different grass species and analysis of proteolytic stability. J. Allergy Clin. Immunol. 107 (5), 856–862. http://dx.doi.org/10.1067/mai.2001.114114.
Petersen, A., Dresselhaus, T., Grobe, K., Becker, W.M., 2006. Proteome analysis of maize pollen for allergy-relevant components. Proteomics 6 (23), 6317–6325. http://dx.doi.org/10.1002/pmic.200600173.
Phillips, J.W., Bucholtz, G.A., Fernandez-Caldas, E., Bukantz, S.C., Lockey, R.F., 1989. Bahia grass pollen, a significant aeroallergen: evidence for the lack of clinical cross-reactivity with timothy grass pollen. Ann. Allergy 63 (6 Pt 1), 503–507.
Pomes, A., Davies, J.M., Gadermaier, G., Hilger, C., Holzhauser, T., Lidholm, J., Lopata, A., Mueller, G.A., Nandy, A., Radauer, A., Chan, S.K., Jappe, U., Kleine-Tebbe, J., Thomas, W.R., Chapman, M.D., van Hage, M., van Ree, R., Vieths, S., Raulf, M., Goodman, R., 2018. WHO/IUIS allergen nomenclature: providing a common language. Mol. Immunol this issue.
Ramirez, D.A., Andrews, C.P., Rather, C.G., Jacobs, R.L., 2015. Responsiveness to timothy grass pollen in individuals without known natural exposure in an allergen challenge chamber. Ann. Allergy Asthma Immunol. 114 (3), 226–232. http://dx.doi.org/10.1016/j.anai.2014.11.006.
Saltabayeva, U., Garib, V., Morenko, M., Rosenson, R., Ispayeva, Z., Gatauova, M., ... Valenta, R., 2017. Greater real-life diagnostic efficacy of allergen molecule-based diagnosis for prescription of immunotherapy in an area with multiple pollen exposure. Int. Arch. Allergy Immunol. 173 (2), 93–98. http://dx.doi.org/10.1159/000477442.
Savi, E., Peveri, S., Incorvaia, C., Dell'Albani, I., Marcucci, F., Di Cara, G., Frati, F., 2013. Association between a low IgE response to Phl p 5 and absence of asthma in patients with grass pollen allergy. Clin. Mol. Allergy 11 (1), 3. http://dx.doi.org/10.1186/1476-7961-11-3.
Scala, E., Alessandri, C., Bernardi, M.L., Ferrara, R., Palazzo, P., Pomponi, D., ... Mari, A., 2010. Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. Clin. Exp. Allergy 40 (6), 911–921. http://dx.doi.org/10.1111/j.1365-2222.2010.03470.x.
Seidel, D.J., Fu, Q., Randel, W.J., Riechler, T.J., 2008. Widening of the tropical belt in a changing climate. Nat. Geosci. 21–24.
Shen, H.D., Wang, S.R., Tang, R.B., Chang, Z.N., Su, S.N., Han, S.H., 1988. Identification of allergens and antigens of Bermuda grass (*Cynodon dactylon*) pollen by immunoblot analysis. Clin. Allergy 18 (4), 401–409. Retrieved from. https://gateway.library.qut.edu.au/login? url=https://search.ebscohost.com/login.aspx? direct=true&AuthType=ip,sso&db=afh&AN=16211225&site=ehost- live&scope=site.
Smith, P.M., Suphioglu, C., Griffith, I.J., Theriault, K., Knox, R.B., Singh, M.B., 1996. Cloning and expression in yeast *Pichia pastoris* of a biologically active form of Cyn d 1, the major allergen of Bermuda grass pollen. J. Allergy Clin. Immunol. 98 (2), 331–343 Retrieved from.
Smith, P.M., Xu, H., Swoboda, I., Singh, M.B., 1997. Identification of a Ca2+ binding protein as a new Bermuda grass pollen allergen cyn d 7: IgE cross-reactivity with oilseed rape pollen allergen Bra r 1. Int. Arch. Allergy Immunol. 114 (3), 265–271.
Stintzi, A., Heitz, T., Prasad, V., Wiedemann-Merdinoglu, S., Kauffmann, S., Geoffroy, P., ... Fritig, B., 1993. Plant 'pathogenesis-related' proteins and their role in defense against pathogens. Biochimie 75 (8), 687–706.
Su, S.N., Shu, P., Lau, G.X., Yang, S.Y., Huang, S.W., Lee, Y.C., 1996. Immunologic and physicochemical studies of Bermuda grass pollen antigen BG60. J. Allergy Clin. Immunol. 98 (3), 486–494.
Suphioglu, C., Ferreira, F., Knox, R.B., 1997. Molecular cloning and immunological characterisation of cyn d 7, a novel calcium-binding allergen from Bermuda grass pollen. FEBS Lett. 402 (2-3), 167–172.
Swoboda, I., Grote, M., Verdino, P., Keller, W., Singh, M.B., De Weerd, N., ... Spitzauer, S., 2004. Molecular characterization of polygalacturonases as grass pollen- specific marker allergens: expulsion from pollen via submicronic respirable particles. J. Immunol. 172 (10), 6490–6500 (Baltimore, Md. : 1950).
ThermoFisher, 2012. Allergen Components, ImmunoCAP Allergen Information. ThermoFisher Scientifc, Sweden. http://www.inter-2t.phadia.com/en/Products/Allergy-testing- products/ImmunoCAP-Allergens/Allergen-components-list/.
Timbrell, V.L., Riebelt, L., Simmonds, C., Solley, G., Smith, W.B., McLean-Tooke, A., ... Davies, J.M., 2014. An immunodiagnostic assay for quantitation of specific IgE to the major pollen allergen component, pas n 1, of the subtropical Bahia grass. Int. Arch. Allergy Immunol. 165 (4), 219–228. http://dx.doi.org/10.1159/000369341.
Walker, S.M., Varney, V.A., Gaga, M., Jacobson, M.R., Durham, S.R., 1995. Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. Allergy 50 (5), 405–413. http://dx.doi.org/10.1111/j.1398-9995.1995.tb01170.x.
Wang, T., Chen, Y., Tabuchi, A., Cosgrove, D.J., Hong, M., 2016. The target of beta-expansin EXPB1 in maize cell walls from binding and solid-state NMR studies. Plant. Physiol. 172 (4), 2107–2119. http://dx.doi.org/10.1104/pp.16.01311.
Weber, R.W., 2003. Patterns of pollen cross-allergenicity. J. Allergy Clin. Immunol. 112 (2), 229–239. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1289772.
White, J.F., Bernstein, D.I., 2003. Key pollen allergens in North america. Ann. Allergy Asthma Immunol. 91 (5), 425–435. quiz 435-426, 492. Retrieved from http://www.ncbi.nlm.nih.go v/entrez/query.fcgi? cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14692424.
White, J.M., Majidi, A., Nasar, S.A., Sweeney, M., White, R.S., 2009. Characterisation of the group I allergen of Bahia grass pollen. Open. Allergy J. 27–29.
Wu, Y., Meeley, R.B., Cosgrove, D.J., 2001. Analysis and expression of the alpha-expansin and beta-expansin gene families in maize. Plant Physiol. 126 (1), 222–232 Retrieved from.
Yang, S.-Y., You, Y.-J., Chang, Z.-N., 2005. Molecular Cloning of a Low Molecular Weight Allergen Bermuda Grass Pollen.
Yennawar, N.H., Li, L.C., Dudzinski, D.M., Tabuchi, A., Cosgrove, D.J., 2006. Crystal structure and activities of EXPB1 (Zea m 1), a beta-expansin and group-1 pollen allergen from maize. Proc. Natl. Acad. Sci. U. S. A. 103 (40), 14664–14671. http://dx.doi.org/10.1073/pnas.0605979103.
Ziska, L.H., Beggs, P.J., 2011. Anthropogenic climate change and allergen exposure: the role of plant biology. J. Allergy Clin. Immunol doi: S0091-6749(11)01665-4 [pii] 10.1016/j.jaci.2011.10.032.